



Original Research Article

Chemical speciation of binary complexes of Pb(II), Cd(II) and Hg(II) with L-Cysteine in 1,2 propanediol-water mixtures

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ABSTRACT

Equilibrium study on complex formation of L-Cysteine with Pb(II), Cd(II) and Hg(II) has been investigated pH metrically in 1, 2 Propanediol-water mixtures (0-60% v/v) at 303 K and 0.16 mol L⁻¹ ionic strength. The predominant species detected for Pb(II) and Cd(II) are MLH, ML₂H and ML₂ and those for Hg(II) are MLH, ML and ML₂. The appropriateness of experimental conditions is verified by introducing errors intentionally in the concentrations of ingredients. The models containing different numbers of species were refined by using the computer program MINQUAD75. The best-fit chemical models were arrived at based on statistical parameters. The trend in variation of stability constants of the complexes with dielectric constant of the medium is attributed to the electrostatic and non-electrostatic forces. The species distribution and the plausible equilibria for the formation of the species are also presented.

Keywords: Complex equilibria, Chemical speciation, L-Cysteine, 1, 2-Propanediol, Metals

INTRODUCTION

The speciation study of toxic metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behaviour of protein residues with the metal ion. The species refined and their relative concentrations under the experimental conditions represent the possible forms of amino acids in bio fluid. Lead affects every organ of the body, especially the bones and teeth, the kidneys, the nervous, cardiovascular,

immune and reproductive systems [1]. Lead and other heavy metals create reactive radicals which damage cell structures including DNA and membranes [2-4]. Lead interferes with the normal metabolism of calcium, DNA transcription, enzymes that help in the synthesis of vitamin D and enzymes that maintain the integrity of the cell membrane. Lead may also be harmful to the developing immune

system, causing production of excessive inflammatory proteins.

Cadmium enters the environment through volcanic activity and forest fires [5]. Cadmium affects different kinds of organisms, ranging from microbes to humans. Human exposure to cadmium mainly occurs through cigarette smoking, but exposure can also occur through contaminated food, water or air [6]. Cadmium is a known carcinogen to mammals [7]. Cadmium accumulates in plants, where it is detoxified by binding to phytochelatins [8-10], a family of thiol-rich peptides. Mercury affects the immune system, alters genetics and enzyme systems, and damages the nervous systems, and the senses of touch, taste and vision [11, 12].

L-Cysteine [Cys] is a sulfur-containing amino acid and it forms stable complexes with heavier metals according to Hard and Soft Acid Base theory. Cys is widely applied in many fields, for instance, food additives, pharmaceutical industry, feedstuff and cosmetic additives. Currently, four manufacturing methods have been developed to produce Cys. These are acid or alkali hydrolysis of hair, chemical synthesis, microbe fermentation [13] and bioconversion of 2-amino-thiazoline-4-carboxylic acid (DL-ATC) [14]. Bioconversion of DL-ATC by whole-cell biocatalyst, a competent method with advantages of low energy requirement and high molar yield, has been substituted for acid or alkali hydrolysis of hair as a main method for the production of Cys on industrial scale [15].

1, 2-Propanediol also called propylene glycol (PG) has a dielectric constant [16] of 30.2. The dielectric constant of the medium decreases with increase in the mole fraction of PG. Hence, this medium is chosen to study the acido-basic equilibria to mimic the physiological conditions where the concept of equivalent solution dielectric constant for protein cavities is applicable [17]. The present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the

bonding behaviour of the protein residues with the metal ion. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of these amino acids in the biological fluids. Hence Cys is selected for speciation studies of its complexes with Pb(II), Cd(II) and Hg(II) in propylene glycol (PG)-water mixtures. The protonation constants of Cys in PG-water mixtures were reported earlier [18].

EXPERIMENTAL

Materials

Propylene glycol (Merck, Mumbai) was used as received. Aqueous solutions of L-cysteine and sodium nitrate (E-Merck, Germany) were prepared. Metal solutions of Pb(II), Cd(II) and Hg(II) nitrates were prepared. To increase the solubility of Cys and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 M. To assess the errors that might have crept into the determination of the concentrations, the data have been subjected to analysis of variance of one way classification (ANOVA). The strength of alkali has been determined using the Gran plot method [19, 20].

Apparatus

The titrimetric data were obtained with a calibrated ELICO (Model L1-120) pH-meter (readability 0.01) which can monitor the changes in H^+ concentration. The pH meter was calibrated with 0.05 M potassium hydrogen phthalate in acidic region and 0.01 M borax solution in basic region. The glass electrode was equilibrated in a well-stirred PG-water mixture containing inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of PG (0-60.0 % v/v) maintaining an ionic strength of 0.16 M with sodium nitrate at 303.0 ± 0.1 K. The effect of variations in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor [21].

Procedure

For the determination of stability constants of metal-ligand binary species, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with PG-water mixture of equivalent composition as that of titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 ml. Titrations with different ratios (1 : 2.5, 1 : 3.75 and 1 : 5.0 in the case of Pb(II) and Cd(II) and 1 : 7.5, 1 : 8.5 and 1 : 10.0 in the case of Hg(II)) of metal-to-ligand were carried out with 0.4 mol L⁻¹ sodium hydroxide. Other experimental details are given elsewhere [22].

Modelling strategy

The computer program SCPHD [23] was used to calculate the correction factor. By using the pH-metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75 [24] which exploit the advantage of the constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of binary systems, the correction factor and the protonation constants of cysteine are fixed. The variation of stability constants with the dielectric constant of the medium was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

Alkalimetric titration curves in PG-water mixtures revealed that the acido-basic equilibria of L-Cysteine were active in the pH range 2.0-9.0. Based on the active forms of the ligands in this pH range, models containing various numbers and combinations of complex species were fed to MINIQUAD75 along with the alkalimetric titration data. Exhaustive modelling was performed for a typical system (Table 1).

The models indicated better statistics as the number of species was increased, confirming better fit. There was no further improvement in the fit on inclusion of some more species in the model containing PbLH, PbL₂H and PbL₂. This indicates that the final model appropriately fits the experimental data. Such exhaustive modeling was performed for all the systems. The best-fit model was selected using the statistical parameters [25] of the least squares residuals. The final models along with the statistical parameters are given in Table 2.

The results of the best-fit models contain the type of species and overall formation constants. A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of squares of deviations in the concentrations of ingredients at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around zero mean with little dispersion.

For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. Kurtosis is a measure of the peakedness of the error distribution near a modal value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic as well as platykurtic patterns. The values of skewness recorded in the tables are between -0.26 and 1.39.

These data evince that the residuals form part of a normal distribution. Hence, the least-squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-value recorded.

Table 1: Exhaustive modelling of Pb(II)-cysteine complexes in 40% v/v PG-water mixture

Model number	log β_{mlh} (SD)			U_{corr}	Ske- wness	Kur- tosis	χ^2	R-Factor
	111	120	121					
1	13.11(65)	---	---	4.01	-0.04	14.70	102.2	0.01201
2	-----	13.55(44)	---	4.23	-0.80	2.30	112.6	0.0124
3	-----	-----	18.78(32)	4.42	-0.16	3.24	101.3	0.0128
4	-----	14.37(32)	18.81(35)	4.77	-0.19	4.30	52.64	0.0119
5	12.06(54)	-----	19.58(42)	4.62	-0.18	6.25	63.43	0.0226
6	12.88(19)	15.83(42)	-----	3.42	-0.34	5.90	40.52	0.0117
7	12.65(18)	15.70(12)	19.65(15)	1.00	0.08	4.46	14.71	0.0066

$U_{corr} = U/(NP-m) \times 10^8$, where, m = number of species; NP = number of experimental points

Table 2: Best fit chemical models of Pb(II), Cd(II) and Hg(II) - Cysteine complexes in PG-water mixture

v/v% PG	log β_{mlh} (SD)				pH- Range	U_{corr}	Ske- wness	Kur- tosis	χ^2	R- Factor	
	110	111	120	121							
Pb(II)											
0.0		11.38(10)	14.18(6)	18.21(8)	1.7-4.8	11	1.96	-0.26	2.73	21.52	0.0085
10.0		10.44(17)	15.31(11)	17.79(13)	1.8-4.8	91	1.04	0.34	3.45	24.11	0.0051
20.0		11.74(16)	14.75(21)	18.61(18)	1.7-4.8	97	2.57	0.19	4.50	19.35	0.0078
30.0		11.63(18)	15.49(20)	19.38(17)	1.7-4.8	94	2.29	0.16	4.51	20.56	0.0074
40.0		12.65(18)	15.70(12)	19.65(15)	1.9-5.0	77	1.00	0.08	4.46	14.71	0.0066
50.0		12.74(18)	15.81(22)	19.76(19)	1.9-5.0	75	1.10	-0.12	4.38	12.50	0.0063
60.0		12.74(16)	16.82(13)	20.83(14)	1.8-5.0	92	1.28	0.02	4.02	12.73	0.0085
Cd(II)											
0.0		10.44(10)	12.64(6)	17.05(8)	1.8-6.0	88	1.86	0.08	3.14	52.48	0.0079
10.0		10.44(17)	15.31(11)	17.79(13)	1.8-4.25	80	2.63	0.06	3.59	20.67	0.0081
20.0		10.39(16)	16.82(21)	18.81(18)	1.7-4.10	84	2.54	0.04	3.65	5.08	0.0088
30.0		10.97(14)	17.15(17)	19.05(16)	1.8-4.10	82	2.36	0.06	3.62	3.87	0.0094
40.0		11.50(18)	17.56(12)	19.45(15)	1.9-4.20	85	1.03	0.09	4.46	4.57	0.0086
50.0		11.54(18)	17.65(22)	19.48(19)	1.9-4.20	81	1.13	0.13	4.38	5.40	0.0073
60.0		11.85(16)	17.94(13)	19.75(14)	1.8-4.20	86	1.24	0.16	4.43	6.78	0.0095
Hg(II)											
0.0	8.30 (10)	11.42 (6)	13.75 (8)		1.8-6.5	87	1.86	0.07	4.08	32.07	0.0099
10.0	9.20(16)	12.20(12)	14.45(14)		1.8-6.50	90	2.62	0.54	3.59	13.50	0.0097
20.0	9.27(17)	12.23(22)	14.50(17)		1.8-6.50	98	2.34	0.35	3.69	64.37	0.0089
30.0	9.30(13)	12.30(16)	14.62(16)		1.8-6.50	97	2.37	0.10	3.63	21.71	0.0098
40.0	9.29(16)	12.35(14)	15.13(15)		1.8-6.50	82	1.16	1.39	2.74	37.32	0.0092
50.0	9.42(17)	12.40(21)	15.20(19)		1.8-6.50	84	2.42	0.77	3.64	10.41	0.0093
60.0	9.81(15)	12.51(14)	15.25 (14)		1.9-6.50	80	2.46	0.58	3.66	11.05	0.0091

$U_{corr} = U/(NP-m) \times 10^8$, where, m = number of species; NP = number of experimental points

These statistical parameters thus show that the best-fit models portray the metal-ligand species in PG-water mixture.

Effect of systematic errors on best-fit model

In order to rely upon the best chemical model for critical evaluation and application under varied

experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the influential parameters[26] like concentrations of alkali, mineral acid, ligand and metal (Table 3).

Table 3: Effect of errors in influential parameters on Pb(II)-cysteine complex stability constants in 30%v/v PG-water mixture

Ingredient	% Error	Log β (SD)		
		111	120	121
Alkali	0	11.63(18)	15.49(20)	19.38(17)
	-5	10.24(29)	Rejected	Rejected
	-2	10.58(22)	Rejected	20.10(23)
	+2	11.25(24)	14.35(17)	20.20(18)
	+5	11.50(92)	14.54(86)	21.56(88)
Acid	-5	Rejected	Rejected	Rejected
	-2	10.95(23)	14.34(26)	20.79(25)
	+2	11.09(35)	Rejected	20.56(29)
	+5	11.30(52)	Rejected	20.32(82)
	-5	11.60(29)	15.43(72)	19.37(15)
Ligand	-2	11.61(26)	15.40(22)	19.36(17)
	+2	11.60(22)	15.17(15)	19.97(18)
	+5	11.58(22)	15.14(15)	19.96(19)
	-5	11.53(26)	15.15(18)	19.98(18)
Metal	-2	11.52(23)	15.16(18)	19.96(17)
	+2	11.62(22)	15.17(19)	19.97(17)
	+5	11.64(21)	15.18(20)	19.99(18)

The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. Some species are even rejected when errors are introduced in the concentrations. This study confirms the appropriateness of the chosen best-fit models. This study also indicates the relative sensitivities of model parameters.

Effect of solvent

PG is an amphiprotic and coordinating solvent. It is a structure former and it enhances the water structure in PG-water mixtures; hence, it removes water from coordination sphere of metal ions, making them more reactive towards the ligands. As a result, the stability of the complexes is expected to increase. At the same time, it is a coordinating solvent and competes with the ligands for coordinating the metals. This decreases the stability

of the complexes. Hence, the stability of complex is The variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's classical treatment [27] accounts for the electrostatic contribution to the free energy change. According to this treatment, the

expected to either increase or decrease. energy of electrostatic interaction is related to dielectric constant. Hence, the $\log \beta$ values should vary linearly as a function of reciprocal of the dielectric constant of the medium [26] which is observed in the present study (Figure 1).

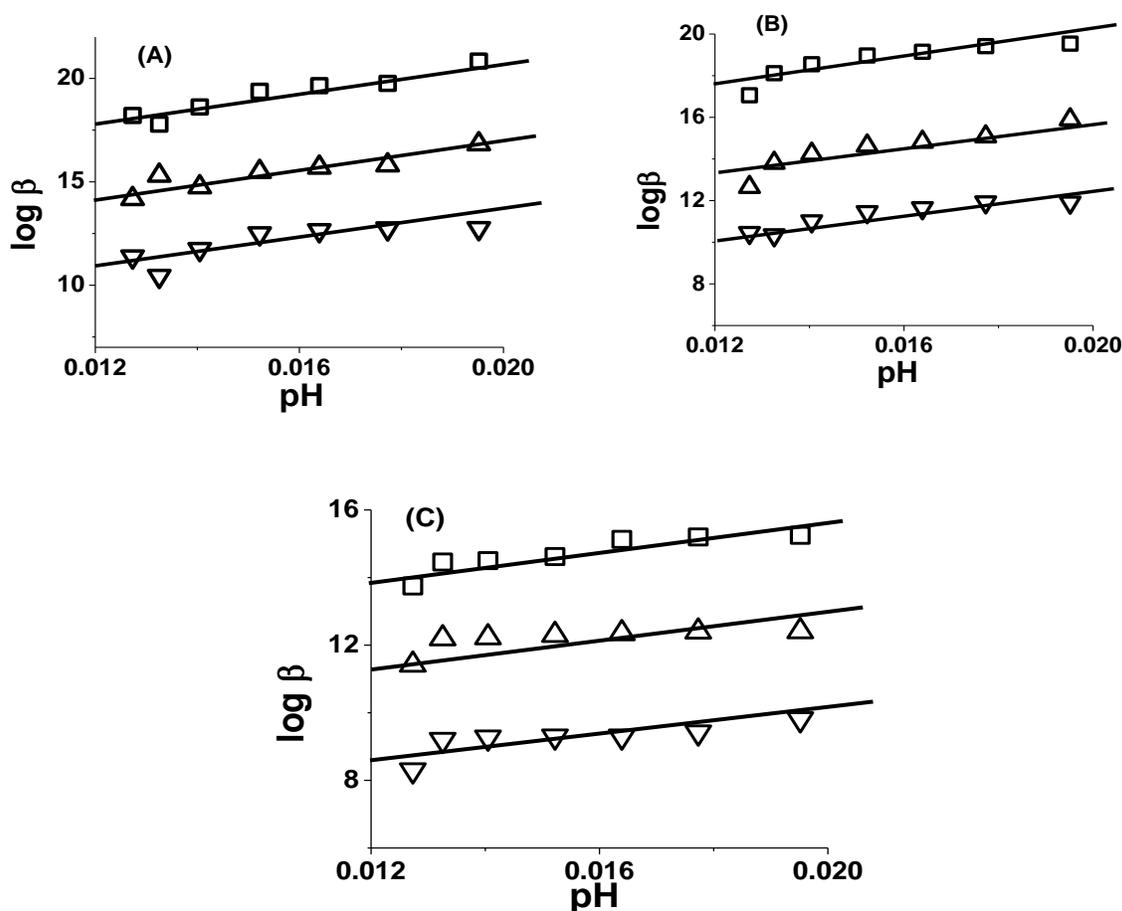


Fig. 1 Variation of stability constant values of metal-cysteine complexes with reciprocal of dielectric constants ($1/D$) in PG-water mixtures at temperature = 303 K, ionic strength = 0.16 M. (A) Pb(II), (B) Cd(II) (∇) $\log 111$, (Δ) $\log 120$, (\square) $\log 121$ and (C) Hg(II) (∇) $\log 110$, (Δ) $\log 111$ (\square) $\log 120$.

The linear variation indicates that electrostatic forces dominate the equilibrium process under the present experimental conditions. The linear increase indicates the dominance of the structure forming nature of PG over its complexing ability. The cation-stabilizing nature of co-solvents, specific solvent-

water interactions, change dispersion and specific interactions of co-solvents with solute (indicated by the changes in the solubility of different species in the aqua-organic mixtures) account for little deviation from the linear relationship.

Distribution diagrams

Cys is a tridentate ligand that has one dissociable (carboxylate group) and two associable amino, thiol group) protons. The different forms of Cys are LH_3^+ , LH_2 and LH^- in the pH ranges 2.0-4, 4.0-10.0 and 10.0-12.0 respectively. Hence, the plausible binary metal-ligand complexes can be predicted from these

data. The present investigation reveals the existence of MLH , ML_2H , and ML_2 for Pb(II) , Cd(II) and MLH , ML and ML_2 for Hg(II) . The formation of various L-Cysteine complex species is shown in the following equilibria. The charges of the species are omitted for simplicity. The species distribution diagrams are shown in Fig. 2.

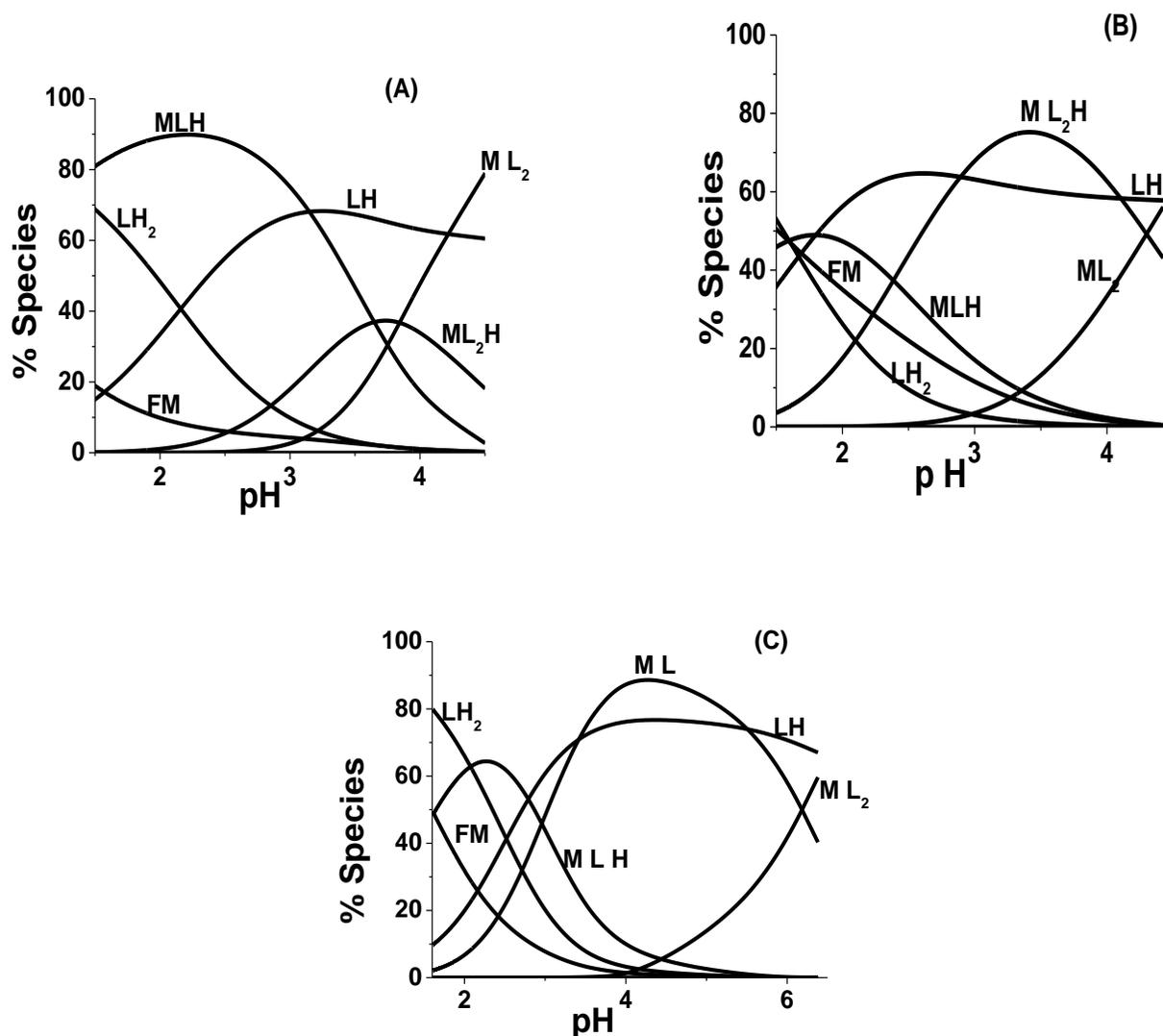
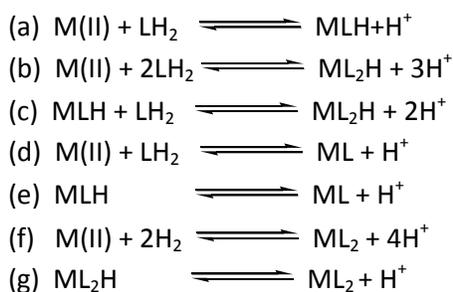


Fig. 2 Distribution diagrams of cysteine complexes in 30 % v/v PG-water mixture at temperature = 303 K, ionic strength = 0.16 M. (A) $\text{Pb(II)} = 0.0999$ mmol, (B) $\text{Cd(II)} = 0.1057$ mmol and (C) $\text{Hg(II)} = 0.0516$ mmol. In all the systems the number of mmols of Cys is 0.50.

At lower pH, MLH species is formed by the interaction of free metal ion with LH_2 form of the ligand (Equilibrium (a)). ML_2H may be formed from

free metal ion and LH_2 (Equilibrium (b)) or from MLH (Equilibrium (c)). At higher pH, ML and ML_2 species are formed from the interaction



of free metal ion with LH_2 (Equilibria d and f, respectively) or by the deprotonation of MLH and ML_2H , respectively (Equilibria e and g). Depending on the active sites in the ligand and the nature of the metal ions, the structures were proposed for the species detected as shown in Fig. 3.

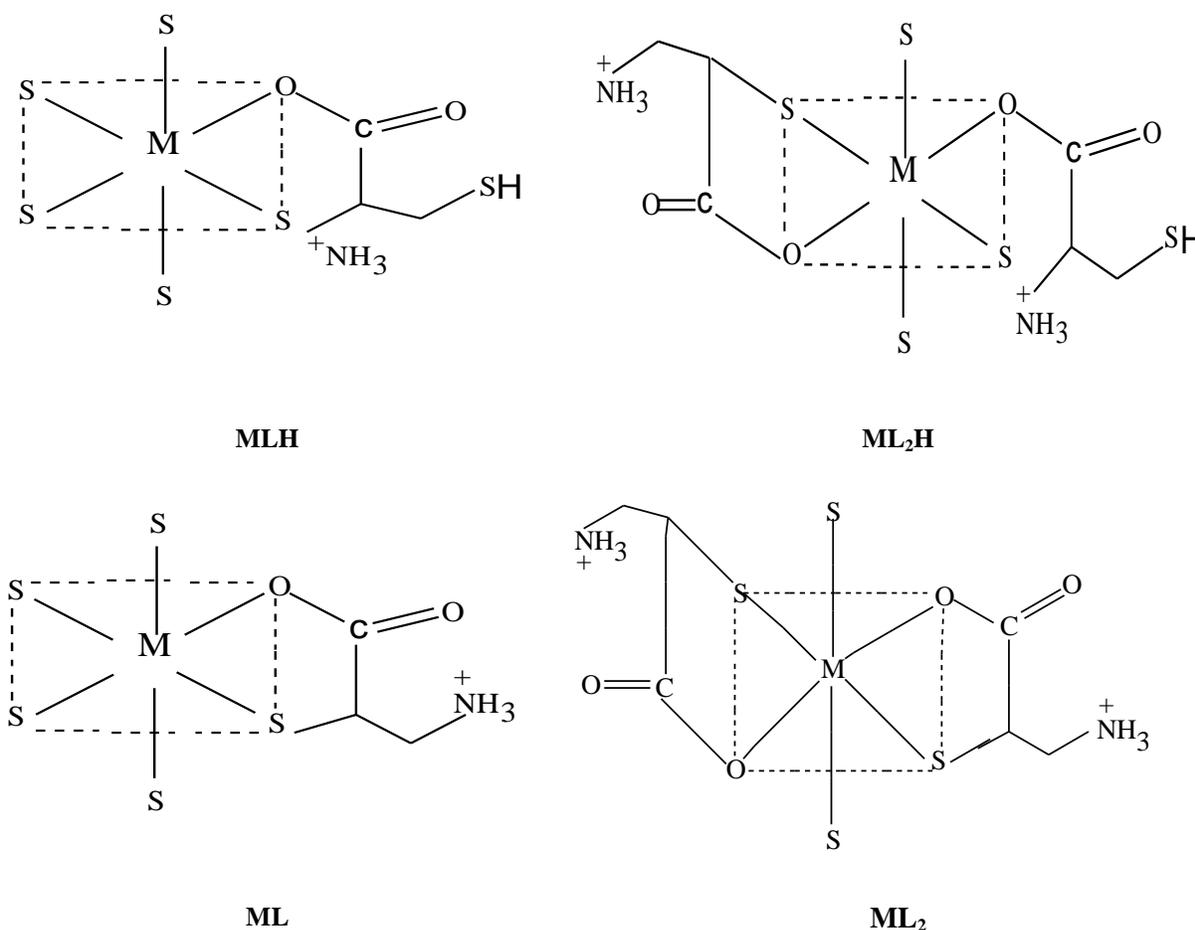


Fig. 3 Structures of binary complexes of Pd(II), Cd(II) and Hg(II) with Cys.

CONCLUSIONS

The present biomimetic studies of metal ion complexes with L-cysteine in PG-water mixtures indicate that the complexes are protonated in acidic pH values. The predominant species detected were MLH , ML_2H , ML and ML_2 due to the interaction of L-cysteine with $Pb(II)$, $Cd(II)$ and $Hg(II)$. The $\log \beta$

values linearly increased with $1/D$ of the medium, indicating the dominance of electrostatic forces over non-electrostatic forces. The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali > acid > ligand > metal.

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